Aust. J. Plant Physiol., 2000, **27**, 159–166

Sustained growth and increased tolerance to glyphosate observed in a C_3 perennial weed, quackgrass (*Elytrigia repens*), grown at elevated carbon dioxide

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Abstract. Although the response of crop plants to rising atmospheric carbon dioxide concentration ([CO_2]) has been well characterized, little is known concerning the long-term growth and/or photosynthetic response of perennial weeds. The growth and photosynthetic characteristics of three cohorts of a perennial C_3 weedy species, quackgrass (*Elytrigia repens* (L.) Nevski) were examined at ~380 μmol mol^{-1} (ambient) and 720 μmol mol^{-1} (elevated) [CO_2] in temperature-controlled greenhouses during 1998 and early 1999. Different cohorts were used to assess the sensitivity of growth, photosynthesis and glyphosate tolerance to elevated [CO_2] for different stages in the life cycle of quackgrass. For the 'old' cohort, planted on Day of Year (DOY) 187, elevated [CO_2] resulted in a consistent stimulation of single leaf photosynthesis, vegetative and whole plant biomass relative to the ambient [CO_2] condition over a 231-d period. Data from the 'intermediate' (DOY 268) and 'young' cohorts (DOY 350) indicated that the stimulation of biomass at the elevated [CO_2] was time-dependent. To determine if the observed stimulation of growth at elevated [CO_2] altered tolerance to chemical weed control, glyphosate [(*N*-phosphonomethyl)glycine] was applied to each cohort and each [CO_2] treatment at rates of 0 (control) and 2.24 kg ai ha^{-1} (sprayed). Tolerance was determined by following the growth and slope of each cohort at the growth [CO_2] treatment for a 28-d period following glyphosate application. For the young cohort, [CO_2] had no affect on glyphosate tolerance; however, an application rate of 2.24 kg ai ha^{-1}, reduced but did not eliminate growth for the intermediate and old cohorts grown at elevated [CO_2]. The basis for increased glyphosate tolerance at elevated [CO_2] for these cohorts was unclear, but was not related to plant size at the time of glyphosate application. Data from this experiment indicate that sustained stimulation of photosynthesis and growth in perennial weeds could occur as atmospheric [CO_2] increases, with a reduction in chemical control effectiveness and potential increases in weed/crop competition.

Keywords: climate change, CO_2 concentration, glyphosate, quackgrass, tolerance.

Introduction

Because of expanded fossil fuel use and deforestation, atmospheric [CO_2] has risen from a pre-industrial concentration of 280 μmol mol^{-1} to a current concentration of ~370 μmol mol^{-1} (Houghton *et al.* 1996). Levels are expected to reach 600 μmol mol^{-1} sometime during this century, even if CO_2 emissions are immediately scaled back to 1990 levels (Bolin 1998). There is concern that an increase in [CO_2] and other trace gases could lead to an increase in global surface temperature with potentially negative consequences for agricultural productivity.

In contrast to any warming effect, the direct physiological effects of elevated [CO_2] are likely to be favorable for most crops. Current ambient [CO_2] is suboptimal for photosynthesis in C_3 crops, and improvements in photosynthetic rate, water-use efficiency, growth and reproductive capacity are anticipated as atmospheric [CO_2] continues to increase. These improvements have, in fact, already been demonstrated in a number of agronomic crops (e.g. Kimball 1983; Kimball *et al.* 1993). However, changes in [CO_2] could significantly influence not only agricultural crops, but the growth, distribution and competitive abilities of associated C_3 weedy species as well (*cf.* Patterson 1993, 1995; Froud-Williams 1996). Significant increases in plant growth and leaf photosynthesis have already been reported for a number of troublesome annual weeds (see Patterson and Flint 1990; Patterson 1993), although less is known regarding the long-term response of perennial weeds. For perennial herbaceous crops, long-term exposure to elevated [CO_2] resulted in complete photosynthetic acclimation, with no further stimulation of growth or biomass in response to increasing [CO_2] (Bunce 1995).

If simultaneous increases in both crop and weedy growth occur in response to future [CO_2], will this necessarily lead to enhanced weed/crop competition? In the US, where labor costs are high, one of the principle means of weed control is through application of herbicides. Among those applied, glyphosate ('Roundup') is particularly noteworthy as a

10.1071/PP99099 0310-7841/00/020159

highly effective phloem-mobile, post-emergent herbicide (Bradshaw *et al.* 1997). Glyphosate's principle mode of action is to inhibit the shikimic acid pathway, which produces aromatic amino acids needed for plant metabolism (Vaughn and Duke 1991). Although there is sufficient available data indicating that the growth of weeds could increase significantly as [CO_2] increases, could the response of C_3 weeds to elevated [CO_2] also alter their response to herbicides such as glyphosate?

At present, the impact of elevated [CO_2] on glyphosate effectiveness is primarily conjectural; however, it should be noted that growth at elevated [CO] could result in anatomical, morphological and physiological changes which could alter herbicide uptake, translocation and overall effectiveness. Increasing [CO_2] can increase leaf thickness, reduce stomatal number and decrease conductance, possibly limiting the uptake of foliar-applied, post-emergent herbicides. Perennial weeds may be of particular interest since increasing [CO_2] could stimulate greater rhizome and tuber growth (Patterson 1993). Greater increases in biomass could result in a dilution of herbicide applied, making weed control more difficult and costly (Patterson 1995).

Given the physiological plasticity of many weed species and their greater genetic diversity relative to modern crops, it is possible that elevated [CO_2] could provide an even greater competitive advantage to weeds with subsequent effects on crop production (Treharne 1989). The goal of the current experiment, therefore, was twofold: (1) to determine if long-term (several months) exposure to elevated [CO_2] can stimulate the photosynthetic and growth responses of a perennial C_3 weed; and, (2) to determine if growth stimulation at elevated [CO_2] can alter the tolerance to a post-emergent herbicide, glyphosate [*N*-(phosphonomethyl)glycine].

Materials and methods

Experimental treatments

Quackgrass (*Elytrigia repens* (L.) Nevski) was chosen as the C_3 perennial to be tested. It is considered a troublesome weed of forage and pasture crops, particularly in the north-eastern and Great Lakes regions of the US (Bridges 1992). All plants were propagated vegetatively from material obtained from Dr Jerry Doll, University of Wisconsin. Cohorts were established in 1998 at three different intervals; Day of Year (DOY)* 187 (6 July), 268 (25 September) and 350 (16 December). These are referred to hereafter in the text as old, intermediate and young, respectively. Cohorts were established by planting a root segment of *ca* 5 cm length into 10-cm diameter (1.2 L) pots. For each [CO_2] treatment, approximately 24 old and 40 intermediate and young plants were established. To test short-term CO_2-induced changes in stomatal conductance, an additional 32 plants for the intermediate cohort were established in the ambient CO_2 glasshouse. As plants grew larger, they were transplanted into larger pots (final volume size of 25 L for the old and 6 L for the intermediate cohort) to avoid root-binding effects. All pots were filled with vermiculite, flushed daily with a complete nutrient solution, and placed so as to minimize mutual shading within a glasshouse.

All experiments were performed in two air-conditioned glasshouses located at the USDA-ARS Climate Stress Laboratory at Beltsville, Maryland. Each glasshouse was 13.5 m^2 in surface area and transmitted 65% of incoming photosynthetic photon flux density (PPFD) with temperature and CO_2 concentration maintained within pre-set limits. Glasshouses were operated to maintain maximum and minimum temperatures of 31 and 17°C, respectively, during the experimental period. Quantum sensors were located near the top of each glasshouse. Dew point temperatures were determined periodically near midday and closely approximated those of outside air. [CO_2] was maintained by a WMA2 infra-red analyser (PP Systems, Haverhill, MA) which injected CO_2 if levels fell below 350 and 700 μmol mol^{-1}, respectively, for each glasshouse. Within a glasshouse, electrical fans continuously circulated air and provided wind speeds of *ca* 0.5 m s^{-1} over leaves. A data logger (21x, Campbell Scientific, Logan UT) recorded PPFD, temperature and [CO_2] in both glasshouses at 30-s intervals. No significant differences with respect to light or temperature were observed between glasshouses (Table 1). [CO_2] treatments were switched between glasshouses twice during the study on DOY 260 and again on DOY 334. Ambient night-time [CO_2] values are higher at this site due in part to low wind speed and stable atmospheric conditions. Intermediate and young cohorts experienced lower PPFD during their growth because of their later planting dates (Table 1).

Gas exchange and growth

Single leaf photosynthesis (determined as *A*, μmol CO_2 m^{-2} s^{-1}) was obtained at 31 and between 55–60 d after planting (DAP) for the old and intermediate cohorts for both CO_2 treatments. At each sampling, assimilation was determined on the uppermost, fully expanded leaf of six plants from each CO_2 treatment. For ambient [CO_2]-grown plants, *A* was determined at both CO_2 concentrations in order to test whether photosynthetic acclimation had occurred. Three d prior to application of glyphosate, additional single leaf measurements were made for 6 plants from the old and intermediate cohorts. Three weeks after glyphosate was applied, photosynthetic measurements were repeated for each cohort for control leaves (non-sprayed) and for leaves which were upright and > 90% green (treated). All measurements were made

Table 1. Average day and night values for temperature, [CO_2] (ambient or elevated) and daytime (PPFD) for the old, intermediate and young cohorts of quackgrass (*Elytrigia repens*) planted on DOY 187, 268 and 350, respectively

Temperature and PPFD values differed by < 1% between glasshouses. Higher ambient night-time [CO_2] is typical for this site. Additional details are given in 'Materials and methods'

Cohort	Time	Temp (°C)	[CO_2] Ambient (μmol mol^{-1})	[CO_2] Elevated (μmol mol^{-1})	PPFD (mol m^{-2} d^{-1})
Old	Day	24.2	383	720	13.4
	Night	18.2	441	731	
Intermediate	Day	22.9	384	727	
	Night	17.7	436	731	
Young	Day	22.2	391	727	
	Night	17.3	432	729	

*Abbreviations used: *A*, single leaf photosynthesis; ai, active ingredient; DAP, days after planting; DOY, day of year; PPFD, photosynthetic photon flux density.

at full sunlight (*ca* 1300–1500 μmol m^{-2} s^{-1}) using a portable open gas exchange system incorporating infra-red CO_2 and water vapor analysers for determining net photosynthetic CO_2 uptake rate and stomatal conductance (CIRAS-1, PP Systems, Haverhill, MA). The vapor pressure deficit surrounding the leaf did not exceed 1.5 kPa during the time of measurement.

Vegetative measurements

To determine whether elevated [CO_2] stimulated growth of quackgrass, shoot growth (i.e. leaves and stems) was sampled at 81, 134 and 163 DAP for the old cohort and at 56 and 83 DAP for the intermediate cohort. Additional whole-plant harvests (leaves, stems and roots) were made at 203 and 231 DAP and at 122, 136 and 150 DAP for the old and intermediate cohorts, respectively, as part of the glyphosate tolerance assessment (see below). Dry weights were obtained separately for leaves, stems and for the later harvests, roots. Leaf area was determined photometrically with a leaf area meter (Li-Cor Corp., Li-3000, Lincoln, NE). All plant material was dried at 65°C until weight was constant and dry weights obtained.

Tolerance to glyphosate was determined based on changes in biomass following glyphosate application. One h prior to spraying and at 14 and 28 d after glyphosate was applied, 8 plants from each cohort, [CO_2] treatment and glyphosate concentration were separated into leaves, stems and roots and harvested as described above. For the old cohort, 8 plants were harvested only at 28 d after spraying. Leaf area was only obtained on upright, green leaves. If dry weight did not significantly increase over the 28-d period following glyphosate spraying, plants were considered to have died.

Glyphosate application

Solutions of commercial glyphosate ('Roundup', Monsanto Agricultural Products Co., St. Louis, MO) were applied to all three cohorts of quackgrass (old, intermediate and young) on 25 January at spray rates of 0 (control) and 2.24 kg active ingredient (ai) ha^{-1} (i.e. 2 quarts of Roundup per acre, the recommended rate for established quackgrass) using a pressurized back-pack sprayer with TeeJet 8003_E nozzles (Spraying Systems Co., Weaton, IL). Sixteen plants per [CO_2] treatment were sprayed with each glyphosate concentration. To prevent [CO_2]-induced changes in stomatal conductance which could, potentially, influence glyphosate uptake, herbicide applications were performed within the respective glasshouses to maintain near-normal ambient and elevated CO_2 levels (372 and 701 μmol mol^{-1}, respectively, when averaged for each glasshouse on the 25 January application date). To determine if stomatal conductance did, in fact, influence glyphosate uptake, an additional 32 plants from the intermediate cohort (16 for each glyphosate concentration) were moved from the ambient to the elevated glasshouse 2 h prior to glyphosate application. Prior to application, stomatal conductance was determined for six plants from each CO_2 treatment (i.e. six ambient-grown and measured, six elevated-grown and measured and six-ambient grown, elevated-measured) for the intermediate cohort using the portable open gas exchange system described previously. After glyphosate application at elevated [CO_2], the ambient grown plants were returned to the ambient glasshouse.

Statistics

The effect of elevated [CO_2] on growth and gas exchange was analysed separately for the old and intermediate cohorts. In addition, the effect of elevated CO_2 on gas exchange was analysed with and without glyphosate application for all thee cohorts, and as a function of the [CO_2] present during the time of spraying. For the sprayed plants, growth rate was considered positive for a given [CO_2] treatment if plant dry weights significantly increased over the 28-d period (two-way ANOVA, time and [CO_2]) and if the slope of the linear regression for changes in biomass following spraying was significantly greater than zero during the 28-d period (confidence intervals for regression coefficient). Unless otherwise stated, significant differences for a given variable as a function of [CO_2] treatment were expressed at the $P < 0.05$ level for a given cohort.

Results

Stimulation of photosynthesis and growth in quackgrass at elevated [CO_2]

For both the old and intermediate cohorts, growth at the elevated [CO_2] resulted in significant stimulation of single leaf photosynthesis at 31 and again at 55–60 DAP relative to photosynthesis rates of single leaves grown and measured at the ambient [CO_2] (Table 2). Differences in the photosynthetic response of single leaves determined at the growth [CO_2] (390 and 720 μmol mol^{-1}) and at a common measurement value of 720 μmol mol^{-1} indicated photosynthetic acclimation for the intermediate cohort. A similar comparison for the old cohort showed a reduction in photosynthesis for leaves grown and measured at the elevated [CO_2] at both sampling dates, but the differences were not significant (Table 2).

A significant [CO_2]-induced increase in shoot biomass was observed for all three harvest dates for the old cohort (Table 3). Although overall shoot growth was stimulated, leaf biomass and area showed the greatest sensitivity to elevated [CO_2] (Table 3). For the intermediate cohort (planted later in the year), elevated [CO_2] had no effect on vegetative biomass or its components at 56 DAP, but did have a significant effect on leaf area and top growth by 83 DAP (Table 3).

Response to glyphosate

Glyphosate was applied on 25 January 1999, 40 DAP for the young cohort and 40 and 39 d from the previous vegetative harvests for the old and intermediate cohorts, respectively. For all cohorts, therefore, application of glyphosate occurred at a time of active plant growth (or regrowth). Because glyphosate inhibits aromatic amino acid biosynthesis in the

Table 2. Average rates of single leaf photosynthesis (determined as *A*, μmol CO_2 m^{-2} s^{-1}) for the old and intermediate cohorts of quackgrass

Rates were determined at the growth CO_2 concentration (*ca* 390 and 720 μmol mol^{-1}) and at a measurement CO_2 value of 720 μmol mol^{-1} for the ambient grown CO_2 treatment. Measurements were take for the last fully expanded leaf at full sunlight (1300–1500 μmol m^{-2} s^{-1}). For each DAP and cohort, *A* was analysed using an one-way ANOVA. Different letters within a row indicate significant differences as a function of measurement [CO_2] ($P < 0.05$, least square means, $n = 6$)

Cohort	DAP	390/390	390/720	720/720
		(μmol CO_2 m^{-2} s^{-1})		
Old	31	19.5 b	31.1 a	29.3 a
	60	17.8 c	29.6 a	27.0 a
Intermediate	31	15.6 c	32.9 a	28.0 b
	55	20.8 c	29.3 a	24.6 b

Table 3. Average vegetative dry weights (leaves and stems) and leaf area for the old and intermediate cohorts of quackgrass (*Elytrigia repens*) grown at ambient and elevated [CO_2]

* indicates a significant difference in dry weight or leaf area as a function of [CO_2] for a given harvest date and cohort. Values for the latter harvest dates reflect vegetative regrowth since the previous harvest. ($P < 0.05$, one-way ANOVA, $n = 24$). Data are weight per plant. Additional details are given in 'Materials and methods'

Cohort	Harvest date	[CO_2]	Leaf area (cm^2)	Leaf wt (g)	Stem wt (g)	Total wt (g)
Old	81 DAP	Amb.	5298	17.7	6.0	23.6
		Elev.	8706*	30.3*	9.3*	39.6*
	134 DAP	Amb.	6292	21.1	7.1	28.2
		Elev.	8872*	30.9*	9.4	40.3*
	163 DAP	Amb.	3101	10.4	3.5	13.9
		Elev.	4144*	14.4*	4.4	18.9*
Intermediate	56 DAP	Amb.	759	2.5	0.9	3.3
		Elev.	820	2.7	1.0	3.7
	83 DAP	Amb.	667	2.2	0.7	2.9
		Elev.	859*	2.8	1.1	3.9*

shikimic acid pathway, active growth is necessary for maximum efficacy of the herbicide (Bradshaw *et al.* 1997).

Elevated [CO_2] *per se* did not result in a significant increase in whole-plant dry weight for the control (non-sprayed) young cohort during the 28 d following spraying, although it is clear that differences in plant dry matter between [CO_2] treatments were increasing as a function of time (Fig. 1). Applying glyphosate at 2.24 kg ai ha^{-1} (100% of the recommended rate) resulted in plant death for this cohort, with no effect of [CO_2] on glyphosate tolerance (Fig. 1).

In contrast, elevated [CO_2] resulted in a significant stimulation of growth and whole-plant biomass for the control (non-sprayed) intermediate cohort (Fig. 2). Glyphosate application resulted in no further stimulation of biomass (and eventual plant death) for the ambient [CO_2]-grown quackgrass in the 28 d after spraying (Fig. 2). Ambient-grown plants from the intermediate cohort which had been moved to the elevated [CO_2] glasshouse for glyphosate application showed the same absence of growth as plants which had been grown and sprayed at ambient [CO_2] (data not shown). However, elevated [CO_2]-grown plants did demonstrate a positive increase in growth by 28 d after spraying (Fig. 2).

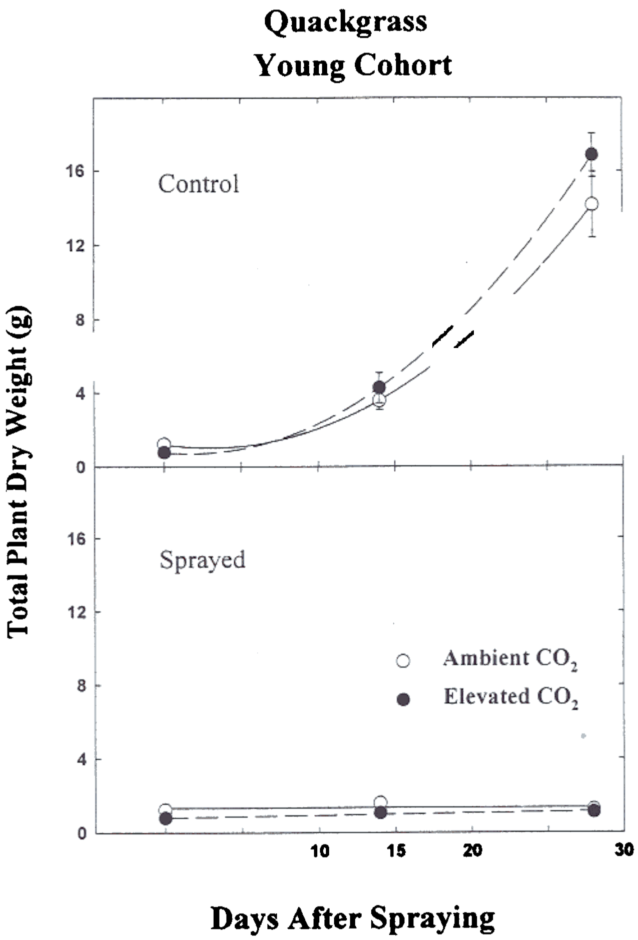
For the old cohort, elevated [CO_2] also resulted in a significant increase in biomass for the control plants at 0 and 28 d after spraying (Fig. 3). As observed with all previous cohorts, glyphosate applied to the ambient [CO_2] grown plants resulted in loss of growth and plant death. However, as with the intermediate cohort, elevated [CO_2]-grown quackgrass showed a positive increase in total plant biomass during the 28 d after glyphosate application. For both the intermediate and old cohorts, green leaf material and leaf photosynthesis were significantly greater for the elevated relative to the ambient [CO_2] treatment 21 d after glyphosate application (Table 4).

As an additional test to determine regrowth potential of glyphosate-treated plants, roots from 2 sprayed plants from each [CO_2] treatment of the old cohort were cut into 3–5 cm-long segments and the segments placed within 30 × 50 × 10-cm flats filled with vermiculite. Flats were placed into the respective [CO_2] glasshouses and checked for regrowth. Regrowth was observed for the elevated [CO_2], glyphosate-treated quackgrass, whereas no regrowth was noted for the ambient [CO_2] grown, glyphosate-treated plants (Table 5).

Discussion

To date, most studies have focused on the short-term (30–45 d) photosynthetic and growth responses of annual weedy species to elevated [CO_2] (Patterson and Flint 1980; Patterson 1986; Patterson *et al.* 1988; Tremmel and Patterson 1994; Bunce 1997; see Poorter 1993 for a review). A single previous study had been conducted for quackgrass at elevated [CO_2] demonstrating an overall growth stimulation of 64% over a 32-d period (350 *vs* 700 μmol mol^{-1} CO_2, Tremmel and Patterson 1994). However, the impact of quackgrass on cropping systems can be long-term (several months) due, in part, to its persistence as a perennial weed. Since long-term growth at elevated carbon dioxide can also lead to CO_2 insensitivity or acclimation of photosynthesis and growth, it would be important to characterize the sustainability of the growth response of quackgrass at elevated [CO_2].

For quackgrass, vegetative harvests for the old cohort taken at 81, 134 and 163 DAP and whole-plant harvests taken at 203 and 231 DAP for the unsprayed controls indicate a consistent stimulation of biomass in response to elevated [CO_2] for this cohort during this period. Single leaf photo-



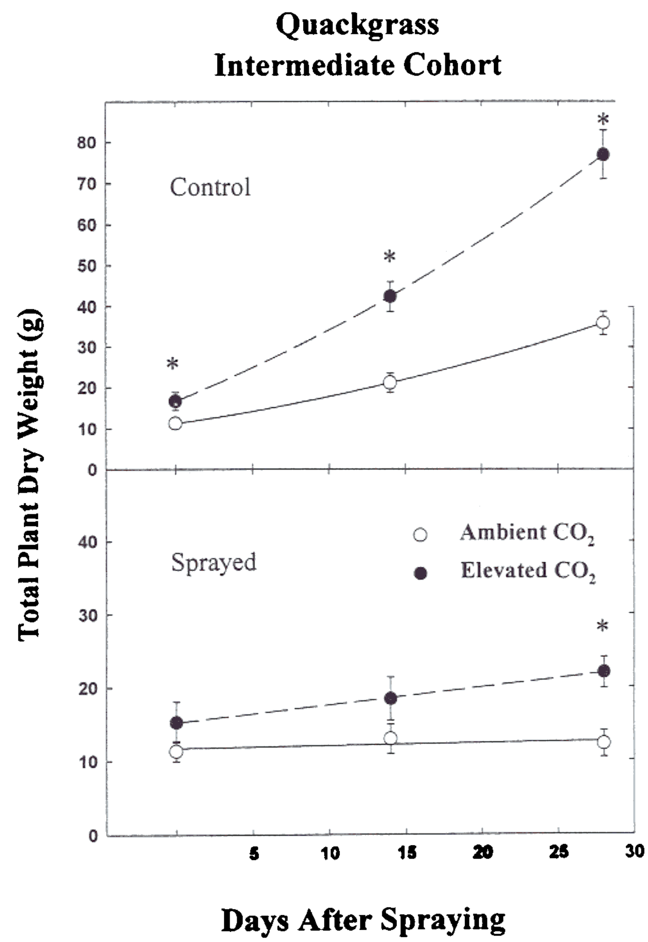


Fig. 1. Change in whole-plant dry weight (± s.e.) for the young cohort (DOY 350) of quackgrass during the 28-d period following application of glyphosate at either 0 (control) or 2.24 kg ai ha^{-1} (100% of the recommenced commercial rate). Plants were grown at either ambient (O, solid line) or elevated (●, dashed line) [CO_2]. Glyphosate was applied on 25 January 1999. For the sprayed plants, growth rate was considered positive for a given [CO_2] treatment if plant dry weights significantly increased over the 28-d period (two-way ANOVA, time and [CO_2]) and if the slope of the linear regression for changes in biomass following spraying was significantly greater than zero during the 28-d period (confidence intervals for the regression coefficient). * indicates a significant difference between elevated and ambient [CO_2] treatments for a given sampling time and application rate, $n = 8$.

Fig. 2. As for **Fig.** but for the intermediate (DOY 268) cohort, $n = 8$.

synthetic rate also increased significantly in response to elevated [CO_2] up to 233 DAP (i.e. Table 4 for the old cohort control). A similar response of biomass and photosynthesis was observed for the intermediate cohort, although no initial stimulation of vegetative biomass was observed at 56 DAP. Only the young cohort did not show enhanced biomass in response to the elevated [CO_2] treatment, although the differences in biomass between [CO_2] treatments appeared to increase over time (e.g. Fig. 1). The reduction in the stimulatory response to [CO_2] for the young and intermediate cohorts could be due to reductions in average PPFD associated with the later planting dates, but this is not clear.

Overall, data from the current study demonstrate that quackgrass can respond to an elevated [CO_2] environment over the long-term (i.e. up to 231 DAP for the unsprayed controls of the old cohort), with significant enhancements in both leaf photosynthesis and plant biomass. This finding is in contrast to the response of other herbaceous agronomic perennials such as alfalfa and orchard grass to elevated [CO_2], where the stimulation of biomass by elevated [CO_2] (as determined by vegetative regrowth) was observed initially, but diminished over time (Bunce 1995).

One of the notable features of quackgrass is an extensive root system. The deepest and most vigorous rootstocks occur in cultivated fields and may extend laterally 3–5 feet (approx. 0.4–0.6 m) (Robbins *et al.* 1970). Roots also act as storage organs and each cord-like rootstock is capable of propagation (Robbins *et al.* 1970). In cultivated fields, dispersal of roots by mechanical means (hoes, cultivation), is one reason why quackgrass is such a pernicious weed. The

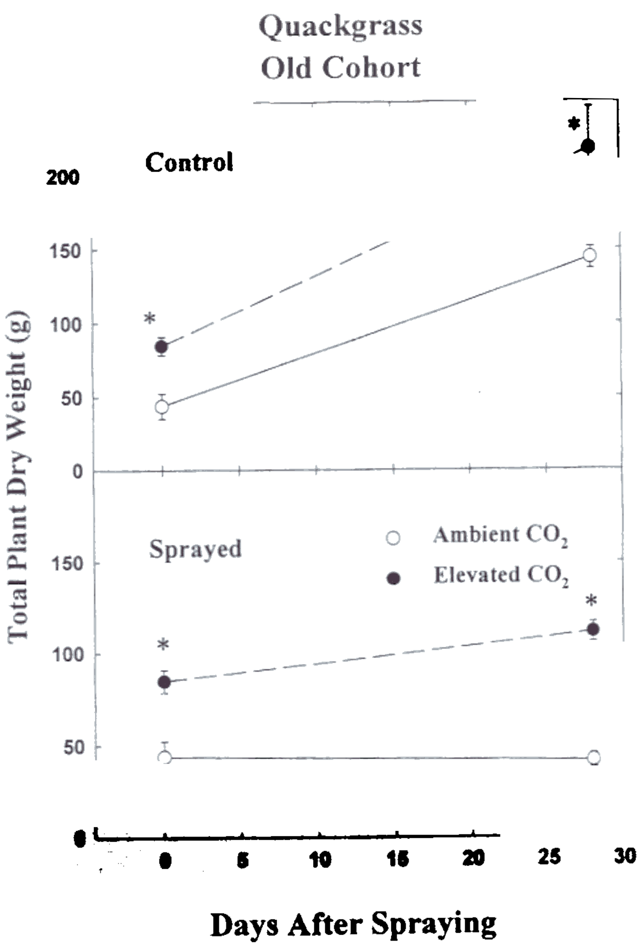


Fig. 3. As for Fig. but for the old cohort, $n = 8$

Table 4. Changes in single leaf photosynthesis (μmol m^{-2} s^{-1}) for quackgrass grown at ambient and elevated [CO_2]

Measurements were obtained 3 weeks after spraying with glyphosate at either 0 (control) or 2.24 kg ai ha^{-1} (100% of recommended rate). Measurements were take for the last fully expanded leaf (control), or any sprayed leaf which was upright and > 90% green (treatment) for the old and intermediate cohorts at full sunlight (~1300 μmol m^{-2} s^{-1}). For each cohort, * indicates a significant difference in A between the elevated and ambient [CO_2] treatments for a given application of glyphosate. 0.0 indicates that green leaves were not available (student's unpaired t-test, $n = 4$)

Cohort	[CO_2]	Glyphosate application: Control (μmol CO_2 m^{-2} s^{-1})	100%
Old	Amb.	15.6	0.0
	Elev.	30.7*	8.4*
Intermediate	Amb.	16.9	0.0
	Elev.	29.4*	7.9*

Table 5. Vegetative regrowth from root segments of glyphosate treated quackgrass (old cohort) grown at either ambient or elevated [CO_2]

Root segments from sprayed plants were planted on 22 February 1999. Regrowth (if present) was harvested on 23 April 1999. During the regrowth period, plants were exposed to their respective growth [CO_2]. Glyphosate was applied on 25 January at a rate of 2.24 kg ai ha^{-1}. A value of zero indicates no regrowth occurred. Data are given on a per plant basis. Quackgrass typically regrows from cut root segments *in situ*

[CO_2]	Leaf area (cm^2)	Leaf wt (g)	Stem wt (g)	Total wt (g)
Amb.	0.0	0.0	0.0	0.0
Elev.	214	1.2	0.5	1.7

presence of a large non-photosynthesizing root sink and a high sink to source ratio could explain, in part, the long-term growth response of quackgrass to elevated [CO_2] (see Bowes 1996). The growth response observed in the current experiment indicates that quackgrass could potentially be a more aggressive competitor in agricultural fields in a future, higher [CO_2] environment.

But the competitive ability of quackgrass with increasing [CO_2] in agricultural systems will also depend in large part on the efficacy of chemical weed control. How will stimulation of growth in quackgrass at elevated [CO_2] affect its response to popular post-emergent herbicides like glyphosate?

In the current study, growth at elevated [CO_2] had no effect on tolerance to glyphosate for the youngest cohort (DOY 350) when applied at 40 DAP. However, elevated [CO_2] did increase tolerance to glyphosate application for the intermediate and oldest cohort. In these cohorts significant regrowth was observed vegetatively for sprayed plants. In addition, old cohort rootstocks grown at elevated [CO_2] showed significant regrowth after being treated with 2.24 kg ai ha^{-1} of glyphosate.

In general, it is recognized that the larger a plant when a post-emergent herbicide is applied, the less effective the herbicide. The relative influence of size on glyphosate tolerance can be determined by plotting the ratio of the dry weight of treated plants to that of the unsprayed control 28 d after glyphosate application as a function of the initial dry weight at the time of spraying. If increased tolerance is simply a function of plant size, then ambient and elevated [CO_2] grown plants should have a similar response. However, in the current experiment, a distinct response was observed for each [CO_2] treatment (Fig. 4). While glyphosate tolerance increased with increasing plant size up to 30% of the unsprayed control for ambient [CO_2] grown plants, no such saturation effect was observed for elevated [CO_2] treated plants. Clearly, data from additional cohorts is needed to fully elucidate glyphosate tolerance as a function of plant size and [CO_2]. However, the current data suggest that dif-

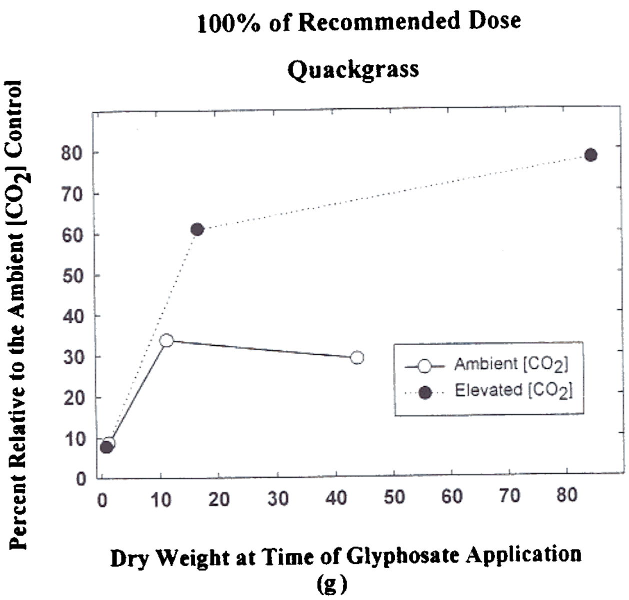


Fig. 4. Relationship between the initial weight of a quackgrass cohort at the time of glyphosate application and the ratio of the dry weight of the sprayed plants to the ambient [CO_2] control (non-sprayed) plants after a 28-d period (as a percent). Increases in the 'percent relative to the control' indicate increasing tolerance to glyphosate.

ferences in glyphosate tolerance may not be simply a function of plant size at the time of spraying.

What is the basis then for altered glyphosate tolerance in quackgrass at elevated [CO_2]? In general, tolerance would be improved if either the amount of herbicide entering the leaf was reduced, or alternatively, the physiological action of the herbicide was impaired in some way. One of the ubiquitous affects of increasing [CO_2] is stomatal closure and reduced transpiration. Presumably this could reduce the uptake of foliar-applied herbicides such as glyphosate. In the current study, a subset of plants grown at ambient [CO_2] from the intermediate cohort were placed in the elevated [CO_2] condition approximately 2 h prior to spraying. This short-term exposure resulted in a 24% reduction in stomatal conductance (420 *vs* 320 mmol m^{-1} s^{-1}) but no change in glyphosate tolerance. However, stomatal conductance for the long-term elevated-grown plants was reduced 45% relative to the ambient grown control (420 *vs* 227 mmol m^{-1} s^{-1}). While these results suggest that glyphosate tolerance is independent of a short-term, direct effect of [CO_2], long-term [CO_2]-induced changes in leaf conductance (e.g. reductions in stomata cm^{-2}, changes in cuticle thickness) could potentially reduce glyphosate absorption. Even if glyphosate absorption is not affected, there are a number of [CO_2]-induced physiological changes which could also alter glyphosate effectiveness. In general, protein content per gram of tissue can be reduced with increasing [CO_2] (Bowes 1996) which could result in less demand for aromatic amino acids. Since glyphosate inhibits aromatic amino acid production through the shikimic acid pathway, this could influence the toxicity of the herbicide. Alternatively, high leaf starch concentrations, which commonly occur in C_3 plants under CO_2 enrichment could interfere with herbicide function (Patterson 1993). However, at present, the issue remains primarily speculative; additional experiments are needed to determine the exact mechanism by which elevated [CO_2] alters tolerance to glyphosate in quackgrass.

It has long been acknowledged that the ongoing increase in atmospheric [CO_2] could lead to increases in commercial crop productivity, but relatively less attention has been given to the response of troublesome agricultural weeds (see Patterson 1995). Data from the current study demonstrate that sustainable growth enhancement of perennial weeds could occur in a future, elevated [CO_2] environment, and that control of quackgrass using chemical herbicides like glyphosate could be altered, especially for established plants. Obviously, quackgrass control could still be achieved if spraying occurs early in the growth cycle, or if additional applications of glyphosate were used; but this could, potentially, alter the economic or environmental costs. While additional field-based data are needed to confirm these observations, sustainability of growth for perennial weeds, and alteration of glyphosate tolerance could have significant consequences for weed/crop competition and production losses in a future, elevated [CO_2] environment.

Acknowledgment

The authors thank Dr J. T. Baker for his comments on the manuscript.

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Manuscript received 29 June 1999, accepted 18 November 1999